

CLAIMS

1. A method of amplifying a target RNA containing a specific base sequence in a sample by an RNA amplification procedure which comprises a step of forming
5 a double-stranded DNA which has a promoter sequence and is capable of being transcribed into an RNA consisting of the specific base sequence or a sequence complementary to the specific base sequence by using the target RNA as the template, a step of forming an RNA transcript consisting
10 of the specific base sequence or a sequence complementary to the specific base sequence by using an RNA polymerase and a step of forming the double-stranded DNA by using the RNA transcript as the template, in the presence of adenosine triphosphate, uridine triphosphate, cytidine
15 triphosphate, guanosine triphosphate and inosine triphosphate as the substrates of the RNA polymerase.
2. The amplification method according to Claim 1, wherein RNA polymerase from phage SP6 is used as the RNA polymerase, and inosine triphosphate is added to a final
20 concentration of from 0.5 mM to 2 mM in the amplification procedure.
3. The amplification method according to Claim 2, wherein the ratio of the final concentration of inosine triphosphate to the final concentration of the other
25 ribonucleoside triphosphates (adenosine triphosphate, uridine triphosphate, cytidine triphosphate and guanosine triphosphate) is from 0.5:1.0 to 1.5:1.0.

4. The amplification method according to Claim 1,
wherein in the amplification procedure, tris-HCl buffer
(pH 8.5-8.9) is present at a final concentration of from
20 mM to 50 mM, magnesium chloride is present at a final
5 concentration of from 12 mM to 20 mM, ribonucleoside
triphosphates (adenosine triphosphate, uridine
triphosphate, cytidine triphosphate and guanosine
triphosphate) are present at a final concentration of 3.5
mM to 5.0 mM, RNA polymerase from phase T7 is present as
10 the RNA polymerase, and inosine triphosphate is present
at a final concentration of from 1.0 mM to 2.7 mM.

5. The amplification method according to Claim 4,
wherein the ratio of the final concentration of inosine
triphosphate to the final concentration of the other
15 ribonucleoside triphosphates (adenosine triphosphate,
uridine triphosphate, cytidine triphosphate and guanosine
triphosphate) is from 0.3:1.0 to 0.7:1.0.

6. The amplification method according to Claim 1,
wherein in the amplification procedure, tris-HCl buffer
20 (pH 8.5-8.9) is present at a final concentration of from
50 mM to 80 mM, magnesium chloride is present at a final
concentration of from 12 mM to 20 mM, ribonucleoside
triphosphates (adenosine triphosphate, uridine
triphosphate, cytidine triphosphate and guanosine
25 triphosphate) are present at a final concentration of 2
mM to 3.5 mM, RNA polymerase from phage T7 is present as
the RNA polymerase, and inosine triphosphate is present

at a final concentration of from 3.2 mM to 4.4 mM.

7. The amplification method according to Claim 6,
wherein the ratio of the final concentration of inosine
triphosphate to the final concentration of the other
5 ribonucleoside triphosphates (adenosine triphosphate,
uridine triphosphate, cytidine triphosphate and guanosine
triphosphate) is from 1.0:1.0 to 1.0:1.5.

8. The amplification method according to Claim 1,
wherein the RNA amplification procedure uses a primer
10 complementary to the specific base sequence and a primer
homologous to the specific base sequence (either of which
is a promoter primer having a promoter sequence for the
RNA polymerase at the 5' end) and is characterized in
that the target RNA is used as the template to form a
15 single-stranded DNA by the action of an RNA-dependent DNA
polymerase, the single-stranded DNA is used as the
template for formation of a double-stranded DNA which has
a promoter sequence and is capable of being transcribed
into an RNA having the specific base sequence or a
20 sequence complementary to the specific base sequence by
the action of a DNA-dependent DNA polymerase, the double-
stranded DNA is transcribed into an RNA transcript in the
presence of the RNA polymerase, and the RNA transcript is
used as the template for the subsequent formation of a
25 single-stranded DNA by the RNA-dependent DNA polymerase.

9. A method of assay of a target nucleic acid which
comprises carrying out the amplification procedure as

defined in Claim 1 in the presence of a probe labeled with a fluorescent intercalative dye, and monitoring the fluorescence intensity of the reaction solution.

10. The method according to Claim 9, wherein the probe
5 labeled with a fluorescent intercalative dye alters its fluorescence upon hybridization with the RNA transcript.